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TITLE: Discovery of Hyperpolarized Molecular Imaging Biomarkers
in a Novel Prostate Tissue Slice Culture Model

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14. ABSTRACT Our goal in year 2 was to use the tissue slice (TSC) NMR compatible bioreactor optimized in year 1 to identify hyperpolarized metabolic biomarkers of prostate cancer presence and aggressiveness. To accomplish this goal my group finished the engineering of a 5 mm bioreactor and acquired hyperpolarized [1-13C]pyruvate data indicating that similar signal to noise and quality data can be achieved with 4 to 5 prostate tissue slices in the 5 mm bioreactor as was acquired from 30-40 tissue slices in the prior 10 mm bioreactor. The time course of viability of the tissue slices prior to and after the bioreactor 5 mm bioreactor study was also established using a live-dead tissue assays performed by the co-PI's Peehl and Ronen on this proposal. We subsequently utilized this optimized 5 mm bioreactor in hyperpolarized [1-13C] MR studies to begin correlating hyperpolarized [1-13C] pyruvate metabolism with pathologic grade (aim 2) and observed a grade dependent increase in the flux of hyperpolarized [1-13C]pyruvate to [1-13C]lactate, with higher levels of lactate in higher pathologic grade cancers. More cancer TSCs need to be studied to demonstrate biologic significance.				
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INTRODUCTION

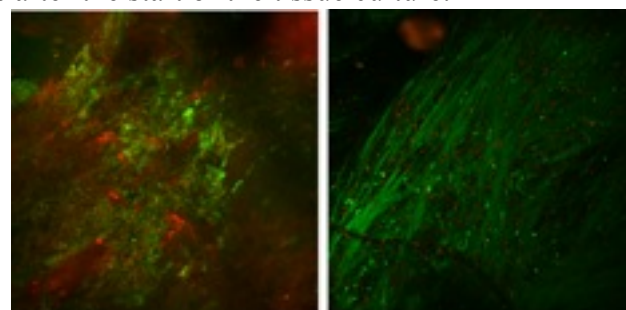
The overall objectives of this synergistic research is to optimize an MRS-compatible, 3D Tissue Culture Bioreactor for use with primary human prostate tissue cultures (TSCs) and use it to identify hyperpolarized molecular imaging biomarkers for improved prostate cancer patient-specific treatment planning and early assessment of response to hormone and chemotherapy. The hypotheses that will be tested in this project are that fresh human prostate tissue slices can be maintained without loss of structure, function or metabolism within a NMR compatible 3-D tissue culture bioreactor for up to three days, and that magnetic resonance spectroscopy studies of these human tissue slices can be used to identify hyperpolarized metabolic biomarkers of prostate cancer presence and aggressiveness and early response to therapy. The goals of this study will be achieved through the following specific aims. The first aim is to optimize conditions for maintaining human prostate tissue slices (TSCs) in an NMR- compatible, 3-D tissue culture bioreactor and to verify the metabolic integrity of TSCs over time. The second aim is to use this new experimental model to compare the metabolism of normal and malignant prostate tissues, and importantly, determine whether hyperpolarized metabolites correlate with pathologic grade (i.e., Gleason grade 3 versus grade 4/5). The third aim is to use NMR to evaluate the response of TSCs to anti-androgen therapy and chemotherapy (Triptolide). ***In the final report below, I summarize the contributions of my lab to this synergistic project.***

BODY

The first Aim was to optimize and validate conditions for maintaining the structure and function of TSCs in an MRS-compatible, 3-D tissue culture bioreactor. As described in the previous years progress reports, my group's research for aim 1 focused on setting up the tissue culture bioreactor procedures and to evaluate varying bioreactor conditions on the structure and function of cultured human prostate tissues. Specifically, we provided significant feed-back on the MR compatible TSC bioreactor being designed by Dr. Kurhanewicz's group, and developed and implemented a procedure to transfer the ^{13}C labeled tissue slices into the NMR compatible bioreactor, while maintaining the metabolic integrity of the TSCs. We also further optimized the tissue culture media developed by Dr. Peehl in order to the maintain pathologic and metabolic viability of prostate tissue slices during the bioreactor studies. The main change made to Dr. Peehl's media was a reduction in glucose concentration used in order to increase the uptake of the ^{13}C labeled pyruvate probe, and an increase in the androgen concentration (R1881) from 10 to 50 nM (see aim 3). We also systematically changed bioreactor conditions (number of tissue slices, thickness of tissue slices, perfusion rate, and oxygenation level) in order to maintain the pathologic and metabolic integrity of benign and malignant human prostate tissues.

As described in the year 2 progress report, my group optimized a histology based live-dead assay (LIVE/DEAD® Viability/Cytotoxicity Kit from Invitrogen) to assess the time course of viability of the tissue slices during the bioreactor studies, and determined its correlation with the energetics of the TSCs in the bioreactor as measured by ^{31}P MRS spectra (i.e. the β -ATP peak area). The graph below shows the average viability of 5 different cases (including normal and cancer) of prostate tissue at various time points, demonstrating the best tissue viability of $\approx 85\%$ at 24 hours, with good viability even at 47 hours after the start of the tissue culture.

Figure 1. Live-dead assay results from prostate tissue slice that was 20% viable (left) versus 80% viable (right).



20% Live

80% Live

Prostate Tissue Slices

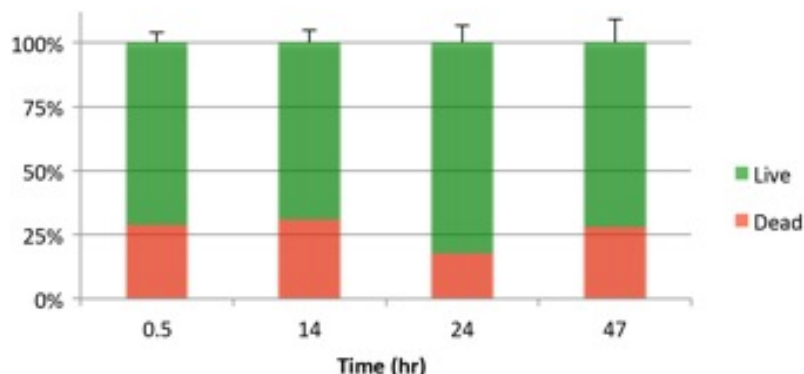


Figure 2. Average viability of 5 different prostate tissue slice cultures at increasing time point after the start of culturing the tissue slices.

In collaboration with Dr. Kurhanewicz's group we demonstrated that ^{31}P spectra obtained during the bioreactor studies, specifically β -ATP, correlated well with % viable tissue as measured by the histologic live-dead assay (**Figure 3**).

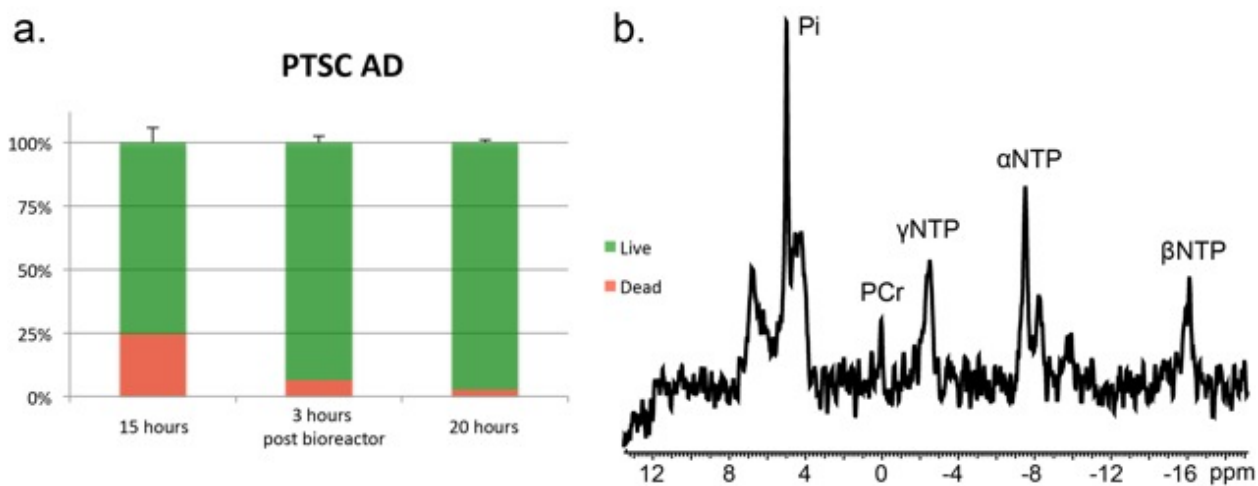


Figure 3. % viability of the 3 prostate tissue cultures at 15 hours post start of tissue 2D culture, and 3 and 20 hours post culturing of the TSC's in the 3D bioreactor (left) and corresponding representative tissue culture tissue slice 13P spectrum obtained after 20 hours in the 5mm 3D MR compatible tissue culture bioreactor. 5 TSC's with $\approx 90\%$ viability correlated with ≈ 50 nmols ATP.

This work is being written up in a manuscript entitled “Optimization and characterization of a 3D human prostate tissue culture bioreactor” which will be submitted to the Journal Magnetic Resonance in Medicine.

Aim 2 was to use the TSC/NMR bioreactor to identify hyperpolarized metabolic biomarkers of prostate cancer presence and aggressiveness. Dr. Kurhanewicz's group utilized the optimized bioreactor conditions to investigate the metabolic reprogramming that occurs with the evolution and progression of prostate cancer (Keshari et al., Prostate, 2013, see “Reportable Outcomes” section). My group was responsible for performing the correlative expression assays of the key pyruvate and lactate transporters (MCT1 and 4) and LDHA expression and LDH activity for benign and prostate cancer tissue slices of increasing pathologic grade as shown in figures 2 and 4 of the Dr. Kurhanewicz's final report. We demonstrated that the increased flux from pyruvate to lactate in the malignant prostate TSCs correlated with both increased expression of monocarboxylate transporters (MCT) and activity of lactate dehydrogenase (LDH).

Aim 3 was to identify hyperpolarized metabolic biomarkers of response to hormone and chemotherapy. As described in Dr. Kurhanewicz's progress report, we found that increasing the concentration of androgen (R1881) from 10 to 50 nM extended the longevity of benign and malignant TSCs within the bioreactor from 1 to 5 days. While reducing androgen to castrate levels in the perfusate (≤ 1.7 nM) resulted in an early (≤ 24 hours) reduction in reduction pyruvate to lactate flux and a later (≥ 24 hours) reduction in tissue ATP. My group demonstrated that the ATP reduction correlated with cell death as evidenced by live dead histology assays (LIVE/DEAD® Viability/Cytotoxicity Kit). As mentioned in Dr. Kurhanewicz's final report the early reduction in pyruvate to lactate flux and subsequent reduction in ATP and cell viability appeared to be most dramatic for benign prostate tissues and decreased with increasing cancer grade. However, more studies are necessary for statistical comparisons, and these studies are ongoing.

KEY RESEARCH ACCOMPLISHMENTS:

- We provided feed-back on the design of the MR compatible TSC, and developed and implemented a procedure to transfer the ^{13}C labeled tissue slices into the NMR compatible bioreactor, while maintaining the metabolic integrity of the TSCs.
- We further optimized the tissue culture media developed by Dr. Peehl in order to the maintain pathologic and metabolic viability of prostate tissue slices during the bioreactor studies and increase uptake of the ^{13}C labeled probe. This optimization resulted in decreased glucose and increased androgen concentration in the media used for the perfusion of the TSCs.
- We optimized bioreactor conditions (number of tissue slices, thickness of tissue slices, perfusion rate, and oxygenation level) in order to maintain the pathologic and metabolic integrity of benign and malignant human prostate tissues, and provide the best signal to noise metabolic data.
- We optimized a histology based live-dead assay (LIVE/DEAD® Viability/Cytotoxicity Kit from Invitrogen) to assess the time course of viability of the tissue slices during the bioreactor studies, and determined its correlation with the energetics of the TSCs in the bioreactor as measured by ^{31}P MRS spectra (i.e. the β -ATP peak area).
- We demonstrated that the best TSC viability was 24 hours after surgery, presumably due to recovery or sloughing of the dead cells at the surface of the tissue slices.
- We demonstrated excellent TSC viability 3 ($\approx 95\%$) and 20 hours ($\approx 98\%$) of being in 3D culture within the MR compatible bioreactor.
- We demonstrated that the increased flux from pyruvate to lactate in the malignant prostate TSCs correlated with both increased expression of monocarboxylate transporters (MCT) and activity of lactate dehydrogenase (LDH).
- We demonstrated that the ATP reduction after reducing perfusate androgen to castrate levels correlated with cell death as evidenced by live dead histology assays.

REPORTABLE OUTCOMES:

- Keshari KR, Sriram R, Van Criekinge M, Wilson DM, Wang ZJ, Vigneron DB, Peehl DM, & Kurhanewicz J Metabolic reprogramming and validation of hyperpolarized (^{13}C) lactate as a prostate cancer biomarker using a human prostate tissue slice culture bioreactor. *Prostate*. 2013; 73(11), 1171-1181.
- Maund, S.L., Nolley, R. and Peehl, D.M. Optimization and comprehensive characterization of a faithful tissue culture model of the normal and malignant human prostate. Submitted to Lab Invest.
- Ronen, S.M. and Kurhanewicz, J. "Optimization and Characterization of a 3D human Prostate Tissue Culture Bioreactor" to be submitted to the Journal Magnetic Resonance in Medicine.

CONCLUSIONS: We have helped successfully achieve a MR compatible robust living human prostate tissue culture bioreactor to study metabolism through optimizing tissue culture bioreactor procedures and conditions, and providing a histologic assessment of tissue viability. We also provided an understanding of the mechanistic rationale for the metabolic changes associated prostate cancer evolution and progression identified by Dr. Kurhanewicz's group through expression and activity assays of key enzymes and transporters involved in pyruvate uptake and metabolism. We believe that this more realistic platform of human prostate cancer in

combination with HP MR will facilitate the identification of clinically translatable biomarkers of prostate cancer presence, aggressiveness, and treatment response.

APPENDICES: N/A